

REMARKS


The Specification has been amended to include sequence identification numbers which were omitted at the time of filing.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made.".

The undersigned hereby states that the paper copy of the Sequence Listing and the computer readable form copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.825(a) and (b), respectively, are the same and contain no new matter. Accordingly, entry of the Sequence Listing into the above-captioned case is respectfully requested.

In the unlikely event that the patent office determines that extensions and/or other relief is required, applicant petition for any required relief including extensions of time and authorize the assistant commissioner to charge the cost of such petitions and/or fees due to our deposit account no. 03-1952 under order no. 286002021121. The assistant commissioner is not authorized to charge the cost of the issue fee to the deposit account.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph [0133] at page 36 has been amended as follows:

Construction of plasmids pRSG32, pBP49, pRSG50: Genes encoding DEBS1+TE (pRSG32), DEBS2 (pBP49) and DEBS3 (pRSG50) were cloned into pET21c (Novagen). The DEBS1+TE gene was cloned as the *NdeI-EcoRI* fragment from pCK12 (6). The DEBS3 gene was cloned as the *NdeI-EcoRI* fragment from pJRJ10 (Jacobsen, J. R., *et al.*, *Biochemistry* (1998) 37:4928). To express the DEBS2 gene, the *BsmI-EcoRI* fragment from pRSG34 (Gokhale, R. S., *et al.*, *Science* (1999) 284:482), which has been used previously to express module 3+TE, was replaced with a *BsmI-EcoRI* fragment encoding module 4. The *EcoRI* site (in bold) was engineered immediately upstream of the stop codon of the DEBS2 gene by modifying the natural sequence to the following: CGGGGGAGAGGACCTGAATTC (SEQ ID NO: 1).

Paragraph [0156] at page 42 has been amended as follows:

Construction of plasmids pBP130, pBP144: The expression vectors pET21c and pET28a were first re-engineered by replacing the *BpuI102I-DraIII* fragments in these vectors with a polylinker possessing the *BpuI102I*, *NsiI*, *PstI*, *PacI* and *DraIII* sites. The DEBS2 gene from pBP49 and the DEBS3 gene from pRSG50 were cloned into the pET21c derivative between the *NdeI-EcoRI* and *NsiI-PacI* sites, respectively, yielding pBP130 (25.5 kb). Thus, pBP130 is capable of expressing the DEBS2 and DEBS3 genes under the control of the same pT7 promoter. Similarly, pBP144 (20 kb) was constructed from the pET28a derivative described above by inserting the *pccAB* genes from pTR132 (Rodriguez, E., and Gramajo, H., *Microbiology* (1999) 145:3109-3119) and the DEBS1 gene into the *NdeI-EcoRI* and *PstI-PacI* sites, respectively. This DEBS1 gene was derived from pRSG32 by replacing the *SpeI-EcoRI* fragment with a fragment amplified from the 3' end of the natural DEBS1 gene using the following oligonucleotides: 5' oligonucleotide: TTACTAGTGAGCTCGGCACCGAGGTCCGGGG (SEQ ID NO:2);

3' oligonucleotide: TTGAATTCGGATCGCCGTCGAGCTCCCGGCCGA (SEQ ID NO:3).

Thus, pBP144 expresses the *pccAB* genes and the DEBS1 gene, each under the control of its own pT7 promoter.

Paragraph [0166] at page 46 has been amended as follows:

Construction of an Expression Vector for the A-T Didomain. An NdeI restriction site was engineered at the start codon of the *rifA* gene using the primers 5'-GCGGCC**CATATG**CGCACCGATCTC-3' (SEQ ID NO: 4) and 5'-AGGGCCCGCTGGCGGGAGAAC-3' (SEQ ID NO: 5) (mutated bases are shown in bold, and the introduced NdeI restriction site is underlined); the amplified 2.5 kb fragment was ligated to linearized pCR-Script (Stratagene) to produce pHu29. The *rifA* gene with the engineered NdeI restriction site at the start codon was then reconstructed in pHu90-1, a derivative of pRM5 (McDaniel, R., et al. (1993) Science 262, 1546-1550), via pHu29, pHu35, pHu50, and pHu51. Flanking restriction sites for PacI and PstI were used to transfer the sequence encoding the loading didomain and part of module 1 from pHu90-1 into a pUC18 derivative to produce pSA2. The loading didomain and module 1 are separated by an ~20 amino acid linker region, delineated by the C-terminal end of the consensus T domain of the loading didomain and the N-terminal end of the consensus ketosynthase domain of module 1 (GenBank accession no. AF040570). To isolate the loading didomain from module 1, a NotI restriction site was introduced into the linker sequence using the primers 5'-ACCGAGACCTGCGGGGCGATCA-3' (SEQ ID NO: 6) and 5'-**GCGGCCGCGACGGCCTGCGTG**-3' (SEQ ID NO: 7) (mutated bases are shown in bold, and the introduced NotI restriction site is underlined); the resulting 0.94 kb fragment encodes from within the loading didomain into the linker region. This amplified fragment was ligated to linearized pCR-Blunt (Invitrogen) to produce pSA4, which was then digested with BamHI and PstI and ligated to pSA2 digested with the same enzymes to generate pSA6. The 1.9 kb NdeI–NotI fragment derived from pSA6 was ligated to NdeI–NotI-digested pET21c (Novagen) to

produce pSA8, an expression vector for the loading didomain with hexahistidine appended to its C-terminus.

Paragraph [0186] at page 46 has been amended as follows:

To engineer a functional fusion between the A-T loading didomain from the rifamycin synthetase and the first module of DEBS, the DNA sequence immediately upstream of the KS domain in DEBS module 1 was modified to read as follows:

CCGGCGAACCG**ATCGCGATCGTCGCGATGG** (SEQ ID NO: 8). The engineered BsaBI site (in bold) was fused to the corresponding naturally occurring BsaBI site between the A-T loading didomain and the first PKS module of the rifamycin synthetase (Figure 6). The resulting fusion was transferred into pBP144 in place of DEBS1, giving rise to pBP165.